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Art. XVI.—*The Specific Identity of Bacillus parabotulinus.*

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Introduction.

In a previous paper the author[1] has given an account of an anaerobic toxin-forming bacillus, which, whilst resembling *B. botulinus*, very closely in certain respects, yet presented certain characters which were deemed sufficient to warrant the organism being regarded as a distinct species, and the name *B. parabotulinus* was therefore proposed for it.

This organism, *B. parabotulinus*, was recovered from bone of an animal that had died of what, in Tasmania, is locally termed Midland Cattle Disease, but the condition commonly occurs also in at least three States of the mainland of Australia. The condition, further, seems to be identical with Lamziekte of South Africa.

A study of *B. parabotulinus* showed that the administration of bacteria-free filtrates of cultures was followed by the same symptoms as those seen in natural cases of the disease, and the organism was therefore considered to be concerned in the etiology of the condition.

On account of the fact that the symptoms induced are those of progressive bulbar paralysis, the condition was described under the title of Toxic Bulbar Paralysis as it was felt that the geographical and other names used in Australia do not convey a meaning expressive of either the cause or the nature of the condition.

It was shown also that in the horse the administration of toxic filtrates of *B. parabotulinus* lead to symptoms identical with those due to botulinus toxin, whether from human or equine sources, and as Forage Poisoning (Cerebro-spinal Meningitis, so-called) in horse has been shown by several workers in America to be due to Botulism, it would seem possible that there are at least three organisms capable of causing Forage Poisoning in Horses, viz., *B. botulinus*, Types A. and B., and *B. parabotulinus*.

Cases of Bulbar Paralysis in both horses and cattle are of not infrequent occurrence in Australia, but, so far as the horse is concerned, no toxicogenic bacilli have as yet been recovered from suspected fodder. In the case of two outbreaks, however, tests of the blood serum from chronic or recovered cases have indicated the presence of specific antitoxins therein. These were from two widely separated outbreaks, and in the one case it would appear that *B. botulinus* Type A. was responsible, in the other case Type B.

1. Journal of Comparative Pathology and Therapeutics, Vol. 35, 1922, part 3. p. 147.

In the case of cattle, it would appear that *B. parabotulinus* is the common cause and not *B. botulinus*, for though the toxin of this latter organism is capable of inducing symptoms of Bulbar Paralysis, it has been shown by Hart and Hayes[2] that the ox is relatively insusceptible, the administration of massive doses of toxin being necessary to cause symptoms.

In order therefore to determine definitely the relationship of *B. parabotulinus* to *B. botulinus*, investigations have been continued and the results of toxin-antitoxin tests are given below, together with a short summary of the other points of difference between these two organisms.

Comparison of B. botulinus (Types A and B) and B. parabotulinus.

(*a*) *Morphological and cultural characters.*

In the earlier paper it was pointed out that while the two types of *B. botulinus* were identical morphologically and culturally (as is well known), *B. parabotulinus* showed certain differences in that (1) it was distinctly larger, (2) it formed a wholly branched colony in solid media, and (3) it failed to show gas formation in glucose media. It was upon these grounds that the organism isolated here was differentiated from *B. botulinus*.

(*b*) *Toxin formation.*

As is well-known, Types A. and B. of *B. botulinus*, both produce powerful toxins, and these toxins are identical in that they both give rise to the same symptoms, but, as has been pointed out by Graham[3] whereas Type A is highly fatal for chickens (in which it induces Limberneck), Type B. toxin is not. Further it was pointed out many years ago by Leuchs, and has been confirmed by others, that the anti-toxin to one type does not protect against the toxin of the other type, and vice versa.

The bacteria-free filtrates of cultures of *B. parabotulinus* likewise induce symptoms identical with those due to botulinus toxin, and, though exact determinations of relative susceptibility have not been made, it has further been shown that cattle and horses possess much the same susceptibility to these filtrates of *B. parabotulinus* (whereas Hart and Hayes[4] have shown that such is not the case with botulinus toxin). The administration of parabotulinus filtrates to chickens in such experiments as have been as yet performed, indicates their behaviour to be similar to that of the toxin of Type B. *B. botulinus*.

Further work has led to the production of much more powerful filtrates of *B. parabotulinus* than those previously recorded, e.g., of one product subcutaneous injection into a guinea pig at the rate of

2. Journal of the American Veterinary Medical Association, Vol. 57 (10), 1920, p. 638.
3. Journal of Infectious Diseases, Vol. 28, 1921, 317.
4. loc. cit.

0.0005 c.c. per kilogramme body weight led to death in 63 hours, and other products have shown almost as high a titre. Even so it is not believed that the limits of toxicity have been reached, and further enquiry is being made into the factors associated with maximal toxin production.

The evidence presented in our earlier paper that *B. parabotulinus* produced a true toxin was as follow:—

1. The administration of germ-free filtrates lead to symptoms identical with those induced by whole cultures. (These filtrates were obtained after passage of culture through Chamberland F. or Doulton candles, and were tested culturally for sterility.)
2. Such symptoms ensued only after a well marked period of incubation, varying according to dosage and route of administration. This period of incubation was commonly of from 15 hours to several days; even after intravenous inoculation of large doses into guinea-pigs no symptoms were manifested for at least six hours.
3. The administration of small doses of such filtrates sufficed to set up the characteristic symptoms and death.
4. The toxicity of a filtrate was diminshed on heating to 60° C. for 15 minutes and entirely destroying by heating to 80° C. for a like period.
5. The fact that filtrates possessed a toxic property analogous to that of botulinus toxin, i.e., were toxic when administered by the mouth.

It was admitted that as no antitoxin had up till then been prepared against these toxic filtrates, the claim that a true specific exotoxin was present in these cultures could not be wholly substantiated.

Production of Antitoxin.

Repeated attempts have been made to immunise small animals against the toxic filtrates of *B. parabotulinus*. In addition to these two horses have also been employed. The first of these horses had to be discontinued for another cause, and the second horse has received as yet only comparatively few injections; a test of its serum reveals the presence of antibodies, but its serum is as yet of too low a potency for critical experiment.*

At first gradual doses of *unaltered toxin*, starting with a small fraction of a lethal dose, were used, but without success, and the animals (guinea pigs and rabbits) invariably succumbed sooner or later as the doses were increased.

For the next attempt *toxin heated to 60° C. for one hour* was employed for the earlier injections, and by this means, two guinea pigs out of six have been successfully immunised. The remainder of these guinea pigs died during the immunisation process; one of those immunised at one time exhibited marked symptoms of paralysis, from which, however, it gradually recovered; the other likewise showed

* This horse has now been successfully immunised. January, 1923. H.R.S.

slight symptoms, and it is noteworthy that with it the initial injections were very much smaller.

In determining the potency of the toxins employed, the minimal lethal dose (m.l.d.) has been interpreted as the smallest quantity which, administered subcutaneously to a 350 grm. guinea pig, has led to death in less than 48 hours.

The following toxin-antitoxin experiments were performed with the serum from one of these guinea pigs (No. 72). The initial dose for this guinea pig was 1/30th m.l.d. of heated toxin (potency determined after heating); nine injections of heated toxin were given, after which the animal received six injections of non-heated toxin; the last injection, nine days before bleeding, was 40 m.l.d. This guinea pig showed symptoms of paralysis after the third injection (1/10th m.l.d.) in consequence of which treatment was suspended for nearly four months; no symptoms have been shown following the subsequent injections, and at time of killing the animal weighed 900 grms. and was in excellent condition.

In order that cross-immunity experiments might be carried out a polyvalent Botulinus antitoxin has been prepared in a cow (Cow 6) by repeated injection of cultures of both type of *B. botulinus*. The strains used were "B. botulinus A1" obtained from the Lister Institute, through the courtesy of the Director, Dr. C. J. Martin, and "B. botulinus, Type B., No. 40" from Dr. K. F. Meyer's collection, kindly supplied by Dr. Hilda Hempl Heller.

Toxin-Antitoxin Experiments.

In the following experiment guinea pigs of 225 to 270 grms. weight were used, and the test was carried out as follows:—The toxin-anti-

Guinea Pig.	Antitoxin.	Toxin.	Result.
336	Parabotulinus 0·2 cc.	Parabotulinus 50 m.l.d.	Lived.
338	Parabotulinus 0·2 cc.	Botulinus A, m.l.d.	Died second day.
340	Parabotulinus 0·2 cc.	Botulinus B, 1 m.l.d.	Died third day.
337	No antitoxin	Parabotulinus 1 m.l.d.	Died second day.
339	No antitoxin	Botulinus A, 1 m.l.d.	Died second day.
341	No antitoxin	Botulinus B, 1 m.l.d,	Died third day.
342	Botulinus 1 cc.	Parabotulinus 1 m.l.d	Died in 20½ hours.
344	Botulinus 1 cc.	Botulihus A, 2 m.l.d.	Lived.
346	Botulinus 1 cc.	Botulinus B, 2 m.l.d.	Lived.
345	Botulinus 1 cc.	Botulinus A, 5 m.l.d.	Lived.
347	Botulinus 1 cc.	Botulinus B, 50 m.l.d.	Lived.
343	Serum normal guinea pig	Parabotulinus 1 m.l.d.	Died in 22 hours.

Antitoxins.—Parabotulinus, Serum G.p. 72.
Botulinus (polyvalent), Serum of Cow 6, 13/9/22.

Toxins.—*B. parabotulinus* of 11/9/22. M.l.d. 0·004 cc.
B. botulinus Type A., A1 of 30/9/22. M.l.d., 1 cc.
B. botulinus, Type B., B·40 of 21/7/22. M.l.d. 0·01 cc.

toxin mixtures, made up to a constant volume (3 cc.) with saline were placed in conical glasses, the contents thoroughly mixed, incubated for 1¼ hours at 37° C., and then injected subcutaneously.

In other experiments it has been shown that 0.1 cc. of this parabotulinus antitoxin is capable of protecting against at least 50 m.l.d. of toxin. Even the administration of 100 m.l.d. of toxin, with a similar quantity of antitoxin, was followed by but slight symptoms and ultimate recovery.

It will be noted that the m.l.d. of Type A. botulinus toxin is very large; this was a very weak toxin, and though this strain has a low degree of toxicity much more powerful products have at times been obtained. The dose selected as the m.l.d. for Type B. botulinus toxin was really less than 1 m.l.d., and this is to be ascribed to the toxicity of this filtrate having diminished somewhat since its potency was determined. These factors, however, only serve to make the test more crucial.

The following conclusions are drawn from the above:—

(a) That the antitoxins employed, parabotulinus and polyvalent botulinus protect against their homologous toxins.

(b) That parabotulinus antitoxin fails to protect against either type of botulinus toxin, and conversely a polyvalent botulinus antitoxin fails to protect against parabotulinus toxin.

(c) That normal guinea pig serum possesses no antitoxic value against parabotulinus toxin.
(In another experiment it has been shown that normal ox serum affords no protection against botulinus toxin.)

(d) That, from immunity tests alone, there is sufficient evidence to regard *B. parabotulinus* as being distinct from *B. botulinus*.

Other tests, though not so comprehensive as that given above, have given the same results.

Conclusion.

(1) That *B. parabotulinus* produces a true toxin, i.e., a soluble exotoxin obtainable by filtration, producing symptoms only after a definite period of incubation, and capable of inducing the formation of an antitoxin.

(2) That though the toxins of *B. parabotulinus* and of *B. botulinus* are identical in their action, the antitoxin to the one does not protect against the other, and vice versa.

(3) As it has been shown previously that *B. parabotulinus* differs from *B. botulinus* (Types A. and B.), both morphologically and culturally, and is now demonstrated by toxin-antitoxin tests to be distinct, the specific identity of *B. parabotulinus* is claimed.